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Biocompatibility of DIAPES®

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Introduction

Extracorporeal blood treatment for patients suffering from end-stage renal disease (ESRD) depends primarily on the performance of the membrane device. The performance has long been measured in terms of the efficiency of removal of unwanted solutes and excess fluid from the patient. Only in more recent years has there been a progressive acknowledgement of the importance of the interaction between the membrane device and the cellular and soluble components of blood. All the potentially harmful consequences of blood contact with the surface of a dialysis membrane go to define the membrane's bio(in)compatibility. In addition to its potential for causing an inflammatory response, membrane-related bioincompatibility is thought to be implicated in many long-term clinical complications encountered in patients on hemodialysis (HD), thus limiting the benefits of this therapy [1, 2]. It thus appears essential that we develop more and more biocompatible membranes characterized by minimization of negative blood/membrane interactions.

Blood compatibility characteristics of HD membranes are generally attributed only to the choice and properties of the base polymer, the so-called surface property-biological response relationship [3]. This concept, however, often excludes the influence of other constituents used in the membrane-manufacturing process which may affect membrane performance characteristics. DIAPES® is a synthetic polymer, a member of the new polyethersulfone family, recently developed for use in hollow-fiber dialyzers. While it is clearly worth evaluating the removal capacity of DIAPES®, as covered elsewhere in this book, it also appears important to determine the impact of this new membrane on the indices of biocompatibility. Satisfactory results for DIAPES® in terms of blood cell count, complement activation and coagulation activation have been reported [4]. In the present prospective randomized cross-over study we evaluate the influence of

the DIAPES® membrane (both low- and high-flux configurations) as compared to conventional polysulfone on the activation of circulating blood cells and their dynamic interactions during in vivo HD.

Subjects and Methods

Patient Population and Dialysis Procedures

Ten stable ESRD patients (4 women and 6 men) on chronic maintenance HD (4 h thrice weekly) for more than 6 months were included in this study after giving informed consent. Exclusion criteria included cardiac and vascular instability, a history of malignancy or hematologic diseases, a positive history of first-use syndrome, unstabilized erythropoietin dosage, clinical evidence of infection at the time of the study, arteriovenous fistula recirculation >10%, and single-needle dialysis. None of the patients had received any medications known to affect leukocyte or platelet function for at least 3 weeks prior to the study. The mean age of patients was 59 (range 34–72) years, and the mean time on dialysis was 41 (range 15–110) months.

A sterile bicarbonate-based dialysate (Na, 140 mEq/l; K, 2 mEq/l; Ca, 3.5 mEq/l; HCO_3, 38 mEq/l; acetate, 5 mEq/l; Bieffe Medital) was used in all treatments. Dialyzers were prepared by rinsing the blood compartment with 2 liters of saline containing 5,000 IU of standard unfractioned heparin. Heparin was the sole anticoagulant used throughout all treatments with an intermittent infusion. Each patient's individual heparin regimen was maintained during the study period. The blood flow rate was kept at 280 ml/min during dialysis, with a dialysate flow rate of 500 ml/min. The ultrafiltration volume was in accordance with individual patient prescriptions.

Experimental Design and Parameters Studied

Each patient included in this study was treated with first-use hollow-fiber dialyzers containing membranes of polysulfone (Bellco BLS627,1.4 m^2, ETO-sterilized), low-flux DIAPES® (Bellco BLS517SD,1.7 m^2, steam-sterilized), and high-flux DIAPES® (Bellco BLS717SD,1.7 m^2, steam-sterilized). Each of the membranes was used in 3 consecutive treatments, the order being randomized by drawing lots. Blood samples were drawn during the 3rd dialysis session with each dialyzer to assess the impact of the membrane on biocompatibility. Measurements were made from the afferent line before dialysis, at 15 min (T:15), 30 min (T:30), and at the end of the session (T:240). Blood was collected in K_3 EDTA vacutainers, and samples were kept cold before use.

Biocompatibility parameters included: the expression on both neutrophil and monocyte surfaces of adhesion molecules CD11b/CD18, CD15s and P-selectin (CD62P); the expression of activation markers CD62P and CD63 on platelet surface; the neutrophil production of reactive oxygen species (ROS), and the formation of circulating platelet-erythrocyte aggregates. CD11b/CD18 (Mac-1, CR3) is a β_2-integrin that mediates adhesion of leukocytes to endothelial cells and participates in other functions such as complement binding and phagocytosis [5]. CD15s (Sialyl-Lewis × molecule) is a ligand of E-selectin expressed on activated endothelial cells and of P-selectin expressed on the surface of activated endothelial cells and activated platelets [6]. The expression of CD62P on neutrophils and monocytes is indicative of platelet-leukocyte coaggregation [7]. The platelet surface antigens CD62P and

CD63 are specific markers of platelet activation [8]. Platelet-erythrocyte aggregates were identified as the flow cytometric events staining for both glycophorin A, a molecule expressed by erythroid cells, and CD61, a marker of resting and activated platelet.

Whole blood flow cytometry was used to determine biocompatibility parameters. Monoclonal antibodies, preparation of whole blood samples and flow cytometric analysis have been previously described in detail [9–11]. The mean fluorescence intensity (in arbitrary units) was used to characterize the expression of CD15s and CD11b/CD18 molecules on cell surface. Results concerning ROS production were measured as a stimulation index (x-fold increase at indicated time points during dialysis over ROS production at time point 0). The results of CD62P expression on neutrophils and monocytes, of CD62P and CD63 expression on platelets, and of platelet-erythrocyte aggregates are expressed as a percentage of positive cells.

Data Analysis

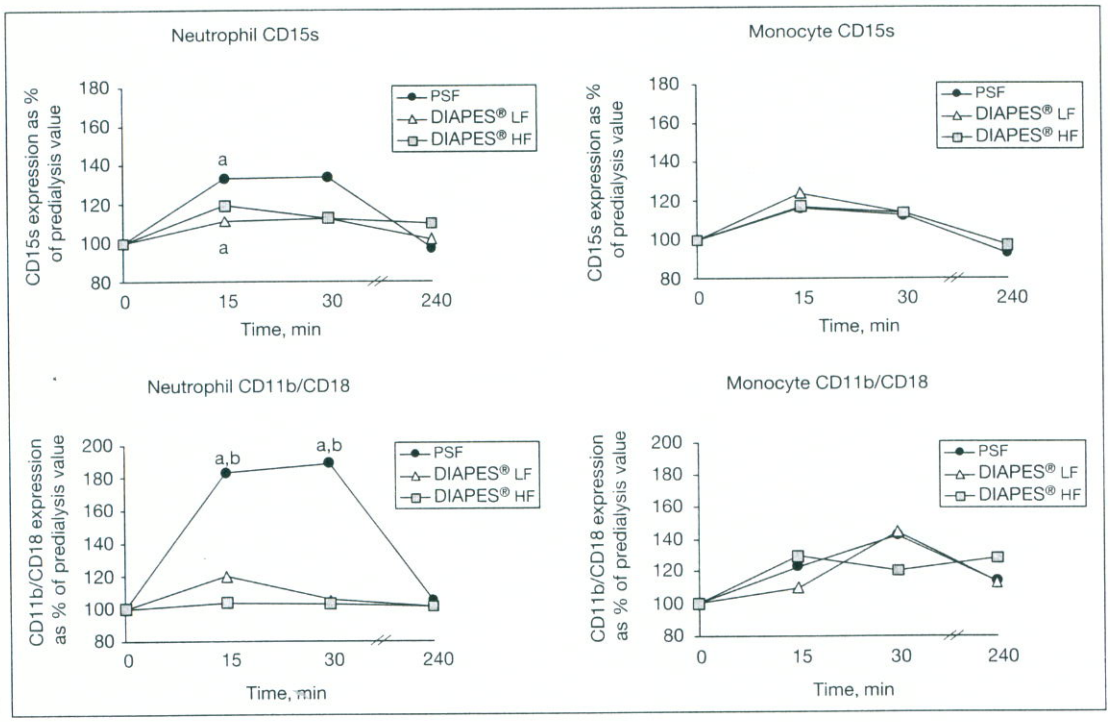
For each patient percentage variations from baseline at 15, 30 and 240 min were computed. The mean percentage variation for each variable was used to provide a representative estimate of the differing efficacy of the three membranes in all patients. Differences between membranes and at different times during dialysis for each membrane were evaluated by repeated measures analysis of variance on ranks due to the skewed distribution of data. Where significant differences were found ($p < 0.05$), differences between individual membranes or times were tested by the Student-Newman-Keuls method. Statistical analyses were performed using the statistical software SigmaStat version 2.0 for Windows (Jandel Scientific Software, San Rafael, Calif., USA).

Results

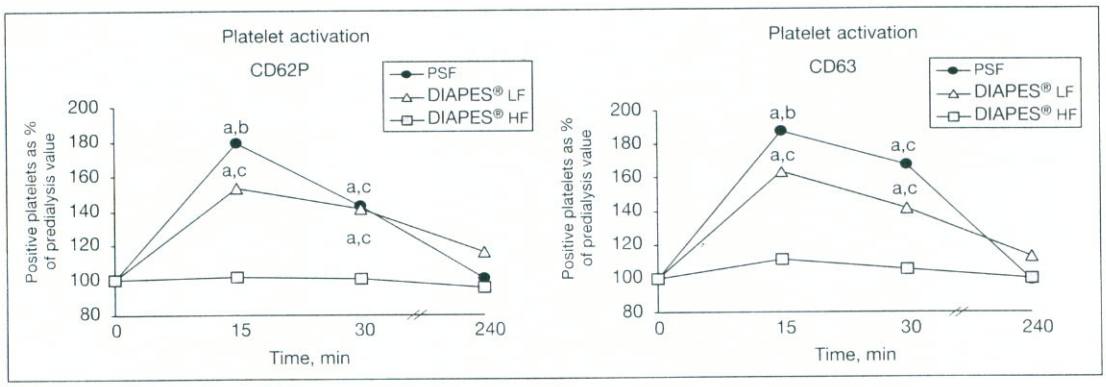
Results for expression of both CD15s and CD11b/CD18 on neutrophils and monocytes are shown in figure 1. Figure 2 shows the results for platelet activation markers CD62P and CD63. Results for platelet-erythrocyte and platelet-neutrophil aggregates are shown in figure 3. As far as platelet-monocyte aggregates are concerned, no significant change was found with any of the membranes (data not shown). Figure 4 shows the results for ROS production by neutrophils.

Discussion

During the HD procedure, circulating blood cells can be activated and also engage in dynamic interplay [9–17]. These phenomena may be important factors behind dialysis membrane bio(in)compatibility since the presence in the patient's bloodstream of a multitude of activated cells and released chemical mediators may be pathophysiologically significant. HD is a repetitive procedure and even mild interactions may, on a chronic basis, lead to adverse side effects.


Neutrophil CD15s
Monocyte CD15s
CD15s expression as % of predialysis value
PSF
DIAPES® LF
DIAPES® HF
a
a
Time, min
Neutrophil CD11b/CD18
Monocyte CD11b/CD18
CD11b/CD18 expression as % of predialysis value
a,b
a,b
Time, min

1


Platelet activation
CD62P
Platelet activation
CD63
Positive platelets as % of predialysis value
PSF
DIAPES® LF
DIAPES® HF
a,b
a,c
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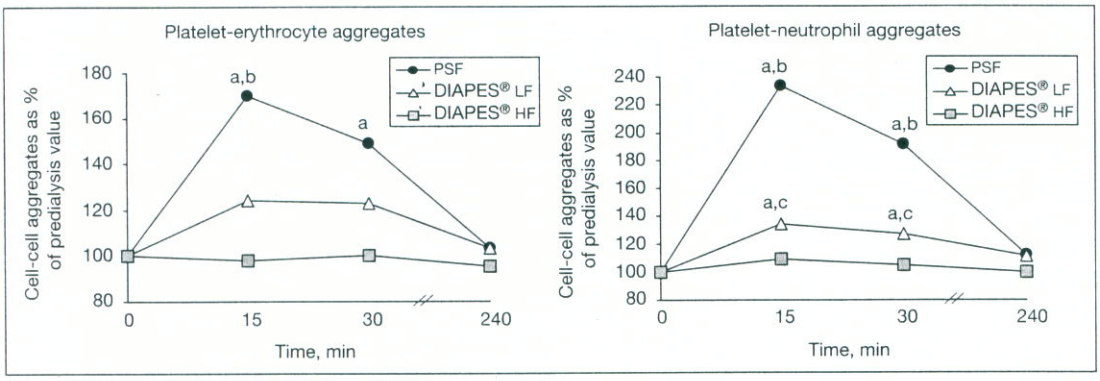
Time, min

2



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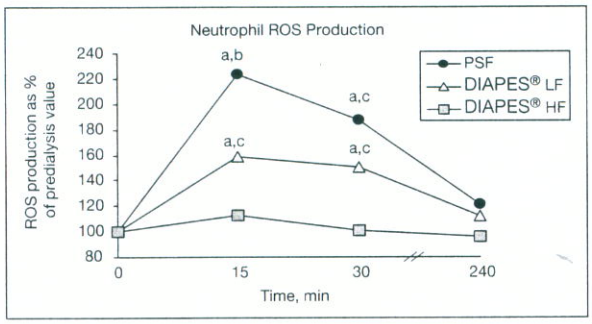

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Fig. 1. Changes in CD15s (Sialyl-Lewis × molecule) and CD11b/CD18 expression on neutrophils and monocytes during hemodialysis with polysulfone (PSF), DIAPES® low-flux (DIAPES® LF) and DIAPES® high-flux (DIAPES® HF). Results are reported as percentage variations from baseline values (set to 100%). [a]Significantly different from baseline; [b]significantly different from DIAPES® LF and DIAPES® HF.

Fig. 2. Changes in activation marker expression on platelets during hemodialysis with polysulfone (PSF), DIAPES® low-flux (DIAPES® LF) and DIAPES® high-flux (DIAPES® HF). Results are reported as percentage variations from baseline values (set to 100%) of platelets positive for CD62P and CD63, respectively. [a]Significantly different from baseline; [b]significantly different from DIAPES® LF and DIAPES® HF; [c]significantly different from DIAPES® HF.

Fig. 3. Changes in platelet-erythrocyte and platelet-neutrophil coaggregates during hemodialysis with polysulfone (PSF), DIAPES® low-flux (DIAPES® LF) and DIAPES® high-flux (DIAPES® HF). Results are reported as percentage variation from baseline values (set to 100%). [a]Significantly different from baseline; [b]significantly different from DIAPES® LF and DIAPES® HF; [c]significantly different from DIAPES® HF.

Fig. 4. Changes in reactive oxygen species (hydrogen peroxide) production by neutrophil population during hemodialysis with polysulfone (PSF), DIAPES® low-flux (DIAPES® LF) and DIAPES® high-flux (DIAPES® HF). Results are reported as percentage variations from baseline values (set to 100%). [a]Significantly different from baseline; [b]significantly different from DIAPES® LF and DIAPES® HF; [c]significantly different from DIAPES® HF.

In the present cross-over study, we investigated the influence of different synthetic polymers (conventional polysulfone, low-flux DIAPES®, high-flux DIAPES®) on the activation of circulating blood cells and their dynamic interactions during in vivo HD. Our results show that there is a considerable difference in the biocompatibility profile of the three membranes tested, dialysis with polysulfone generally being associated with a higher degree of cell reactivity, particularly with regard to platelet activation pathways, than DIAPES® dialyzers. Our results also show that the different flux configuration of DIAPES® dialyzers (low-flux vs. high-flux) results in many similarities but also several significant differences.

The adhesive molecules, CD15s and CD11b/CD18 expressed on the leukocyte surface, facilitate leukocyte-endothelial interactions since their ligands are present on endothelial cells [5, 6]. The study of adhesion molecule expression on leukocytes during HD has been suggested as an important index of biocompatibility [15]. Our data indicate that alterations in the density of these surface molecules involved in cell adherence can occur during HD with synthetic membranes on neutrophils (but not on monocytes) and to a variable degree according to the type of dialyzer used. Our results for CD11b/CD18 on neutrophils agree with previous investigations showing a rapid and prominent increase in the expression of this molecule on neutrophils during HD using biocompatible synthetic membranes [16, 17]. Suggested etiological factors for such upregulation include repeated interaction of neutrophil cells with the membrane or with C3a absorbed onto the membrane [18] and undetectable complement activation by the synthetic membrane [19].

Evidence exists that platelet activation can also occur in patients on HD mainly depending on the flow design of the dialyzer used [20] and on the geometry and composition of the dialysis membrane [12]. The long-term effects of chronic platelet activation induced by HD remain to be defined but an impairment in platelet function following HD has been observed [14]. In addition, the repeated release of platelet-derived substances with significant biological effects [21] might be responsible for some untoward effect of long-term HD, such as accelerated atherosclerosis [22]. Our data indicate a remarkable increase in platelets exposing the activation-dependent antigen CD62P and CD63 during HD with polysulfone, whereas no significant change occurred when patients were dialyzed with a high-flux DIAPES® dialyzer. Results for the low-flux DIAPES® were intermediate though significantly higher than high-flux DIAPES®.

Once activated, platelets have a greater propensity to adhere to other blood cells, resulting in the formation of circulating cellular coaggregates which may be of pathophysiological significance [23, 24]. The study of platelet–leukocyte

interactions has been proposed in recent years as a new parameter to assess the biocompatibility of dialyzer membranes, offering the novel aspect of studying cellular–cellular interactions [for review see, 25]. In the present study, platelet-neutrophil aggregates showed a very prominent increase when using a polysulfone membrane, in keeping with our previous findings [16, 26]. A slight though significant increase was also observed with low-flux DIAPES®, whereas the high-flux DIAPES® membrane did not cause any significant change. Generally coincident with an increase in platelet-neutrophil coaggregates, an increased ROS production by neutrophils was found. Indeed, we have previously demonstrated that once attached to neutrophils during dialysis, activated platelets induce a functional activation of those cells resulting in an increased ROS production [10]. Due to the repeated stimulation of neutrophils during HD, it is thought that the increased ROS production might contribute to some side effects of HD therapy including higher susceptibility to infections [27], vascular injury and atherogenesis [28], and arthropathy due to β_2-microglobulin amyloidosis [29]. Furthermore, we examined the occurrence of platelet-erythrocyte coaggregate formation, a phenomenon that has been observed in patients with sickle cell anemia [24] and more recently in HD patients [11]. Interactions between platelets and erythrocytes markedly enhance several aspects of platelet function in vitro [30] and have been suggested as a possible pathogenic mechanism for the vasculopathy of sickle cell disease [24]. In the present study, platelet-erythrocyte aggregates tended to increase during dialysis sessions with the polysulfone membrane. By contrast, dialysis with either DIAPES® membrane was not associated with any significant change in platelet-erythrocyte circulating levels.

It should be noted that in the present study, in order to overcome variables other than the membrane type, the rheological parameters and heparin dosage were kept constant, patients served as their own controls, and the studies were performed on the same dialysis session of the week using hollow-fiber dialyzers with a similar surface. It appears that each dialysis membrane has multiple and different characteristics that may contribute, more or less favorably, to interactions with blood components. Polysulfone, being hydrophobic by nature, has a low complement-activating potential but can stimulate platelets [31], as also shown here. The DIAPES® membrane was generally associated with a lower degree of cell activation than polysulfone and appears as a valid alternative to that membrane material. In particular, the use of high-flux DIAPES® dialyzers had a negligible effect on most parameters investigated in the present study. Steam-sterilized DIAPES® has been shown to provide optimal and stable performance in high-flux HD for small and large solutes [32]. This dialyzer seems therefore to represent a promising approach for treatment of ESRD by maintenance HD.

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